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September 15, 2004

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APPLICATION NUMBER: 60/501,660
FILING DATE: *September 09, 2003*
RELATED PCT APPLICATION NUMBER: PCT/US04/25026

Certified by

Jon W Dudas

Acting Under Secretary of Commerce
for Intellectual Property
and Acting Director of the U.S.
Patent and Trademark Office



PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EL 961801225 US

INVENTOR(S)					
Given Name (first and middle (if any))		Family Name or Surname		Residence (City and either State or Foreign Country)	
Dusan Zbigniew		Miljkovic Pietrkowsk		San Diego, CA San Diego, CA	
Additional inventors are being named on the _____ separately numbered sheets attached hereto					
TITLE OF THE INVENTION (500 characters max)					
New Nutraceutical Chromium Complexes with Enhanced Biological Activity and Safety					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input checked="" type="checkbox"/> Customer Number: 34284		<input checked="" type="checkbox"/> 34284			
<input type="checkbox"/> Firm or Individual Name					
Address					
Address					
City		State		ZIP	
Country		Telephone		Fax	
ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages 11		<input type="checkbox"/> CD(s), Number _____			
<input type="checkbox"/> Drawing(s) Number of Sheets _____		<input type="checkbox"/> Other (specify) _____			
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76					
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT					
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.		FILING FEE Amount (\$)			
<input type="checkbox"/> A check or money order is enclosed to cover the filing fees.					
<input checked="" type="checkbox"/> The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 502191		80.00			
<input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.					
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input checked="" type="checkbox"/> No.					
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: _____					

Respectfully submitted,

[Page 1 of 1]

Date 9/9/03

SIGNATURE

REGISTRATION NO. 46697

TYPED or PRINTED NAME Martin Fessenmaier

(If appropriate)

Docket Number: 100700.0028PRO

TELEPHONE 714-641-5100

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Pr visional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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**FEE TRANSMITTAL
for FY 2003**

Effective 01/01/2003. Patent fees are subject to annual revision.

☒ Applicant claims small entity status. See 37 CFR 1.27**TOTAL AMOUNT OF PAYMENT (\$)** 80.00**Complete if Known**

Application Number	
Filing Date	August 26, 2003
First Named Inventor	Dusan Miljkovic
Examiner Name	
Art Unit	
Attorney Docket No.	100700.0028PRO

METHOD OF PAYMENT (check all that apply)☐ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None☒ Deposit Account:

Deposit Account Number	502191
Deposit Account Name	Rutan & Tucker

The Commissioner is authorized to: (check all that apply)

☒ Charge fee(s) indicated below ☒ Credit any overpayments☒ Charge any additional fee(s) during the pendency of this application☐ Charge fee(s) indicated below, except for the filing fee to the above-identified deposit account.**FEE CALCULATION****1. BASIC FILING FEE**

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description	Fee Paid
1001 750	2001 375	Utility filing fee	
1002 330	2002 165	Design filing fee	
1003 520	2003 260	Plant filing fee	
1004 750	2004 375	Reissue filing fee	
1005 160	2005 80	Provisional filing fee	80.00

SUBTOTAL (1) (\$) 80.00**2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE**

Total Claims	Extra Claims	Fee from below	Fee Paid
Independent Claims	-20** =	X	
Multiple Dependent	-3** =	X	

Large Entity Fee Code (\$)	Small Entity Fee Code (\$)	Fee Description	Fee Paid
1202 18	2202 9	Claims in excess of 20	
1201 84	2201 42	Independent claims in excess of 3	
1203 280	2203 140	Multiple dependent claim, if not paid	
1204 84	2204 42	** Reissue independent claims over original patent	
1205 18	2205 9	** Reissue claims in excess of 20 and over original patent	

SUBTOTAL (2) (\$)

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)**3. ADDITIONAL FEES**

Large Entity Small Entity

Fee Code (\$)	Fee Code (\$)	Fee Code (\$)	Fee Code (\$)	Fee Description	Fee Paid
1051 130	2051 65			Surcharge - late filing fee or oath	
1052 50	2052 25			Surcharge - late provisional filing fee or cover sheet	
1053 130	1053 130			Non-English specification	
1812 2,520	1812 2,520			For filing a request for ex parte reexamination	
1804 920*	1804 920*			Requesting publication of SIR prior to Examiner action	
1805 1,840*	1805 1,840*			Requesting publication of SIR after Examiner action	
1251 110	2251 55			Extension for reply within first month	
1252 410	2252 205			Extension for reply within second month	
1253 930	2253 465			Extension for reply within third month	
1254 1,450	2254 725			Extension for reply within fourth month	
1255 1,970	2255 985			Extension for reply within fifth month	
1401 320	2401 160			Notice of Appeal	
1402 320	2402 160			Filing brief in support of an appeal	
1403 280	2403 140			Request for oral hearing	
1451 1,510	1451 1,510			Petition to institute a public use proceeding	
1452 110	2452 55			Petition to revive - unavoidable	
1453 1,300	2453 650			Petition to revive - unintentional	
1501 1,300	2501 650			Utility issue fee (or reissue)	
1502 470	2502 235			Design issue fee	
1503 630	2503 315			Plant issue fee	
1460 130	1460 130			Petitions to the Commissioner	
1807 50	1807 50			Processing fee under 37 CFR 1.17(q)	
1806 180	1806 180			Submission of Information Disclosure Stmt	
8021 40	8021 40			Recording each patent assignment per property (times number of properties)	
1809 750	2809 375			Filing a submission after final rejection (37 CFR 1.129(a))	
1810 750	2810 375			For each additional invention to be examined (37 CFR 1.129(b))	
1801 750	2801 375			Request for Continued Examination (RCE)	
1802 900	1802 900			Request for expedited examination of a design application	

Other fee (specify)

*Reduced by Basic Filing Fee Paid

SUBTOTAL (3) (\$)**SUBMITTED BY**Name (Print/Type) Mark A. PessenmaierRegistration No. 46697

(Complete if applicable)

Telephone 714-641-5100Signature [Signature]Date September 9, 2003**WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.**

This collection of information is required by 37 CFR 1.17 and 1.27. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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NEW NUTRACETICAL CHROMIUM COMPLEXES WITH ENHANCED BIOLOGICAL ACTIVITY AND SAFETY

BACKGROUND

Numerous chromium nutraceutical complexes have been described so far in patent and scientific literature. These also include different chromium salts that need not to be real chromium complexes, indeed they may be weak chromium complexes, or simple ionic salts, such as chromium chloride hexahydrate. Only Cr (3+) species are acceptable as nutraceuticals, since all other valence states of Cr are considered unsafe or human health, while Cr (6+) species is highly toxic/genotoxic.

Many chromium (III)-salts represent weak chromium complexes, such as chromium salts with organic bi-tri-and poly-carboxylic acid (such as malic, tartaric and citric acid).

In spite of the relatively large number of known chromium salts and complexes, only few of them have been marketed. The most popular ones are chromium picolinate, and chromium polynicotinate (chromium niacin). Some other Cr (III) complexes, such as Cr-tri-carnosinate are also described in patent literature and are marketed. In addition to those, drum-dried or spray-dried yeast grown in the presence of Cr (III) has been marketed used as a nutraceutical (known as "chromium yeast").

Although there are numerous publications that illustrate efficiency of the marketed chromium supplements to at least some degree, there are also a number of scientific reports where side effects and/or insufficient biological activity have been documented. Several articles describe appreciable genotoxicity of Chromium picolinate (especially in the presence of ascorbic acid that is normally found in all mammalian cells in appreciable concentration).

Very often water insolubility of the used Cr supplements (Cr picolinate, Cr polynicotinate, chromium yeast) is a serious drawback in their application (diminishing their biological activity/bioavailability and/or preventing researchers from testing them in cell culture systems in order to elucidate their biological mechanism of action).

Therefore, there is a constant need to find new chromium compounds/complexes that have higher biological activity/bioavailability, higher safety/less toxicity, sufficient chemical stability and high water solubility.

OUR INVENTION

Our invention relates to new nutraceutical chromium compounds/complexes with enhanced biological activity and safety, and their use. More specifically, through our extended chemical and biological research, we unexpectedly discovered that the optimal (maximal) biological activity of Cr(III) species depend on the chemical stability of their complexes with different ligands directly measured through their association constants or

indirectly measured through the position of their maxima in their visible spectrum (λ_{max}).

Particularly striking examples include different Cr complexes with dipeptide carnosine. CRC5 (chromium-penta-carnosinate), CRC3R (chromium-tri-carnosinate-red) and CRC3V (chromium tri-carnosinate-violet) are the chromium complexes with carnosine (prepared by a reaction of one mole of Cr with five or three moles of carnosine under different experimental conditions (reaction time and temperature as well as slightly acidic, neutral or slightly alkaline medium)).

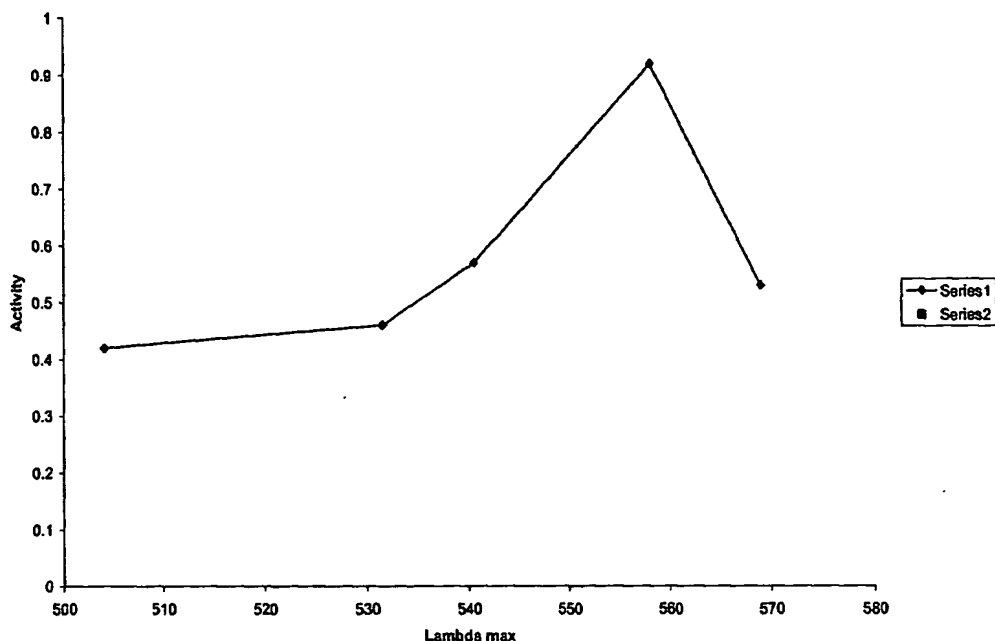
In *in vitro* experiments (on total glucose uptake into L6 muscle cells at 100 nmol concentration), CRC5 increases on average 3.57, CRC3R 4.00 and CRC3V 4.45 fold over control.

In vivo experiments gave much more dramatic differences:

TABLE 1

Diabetic Rats Treated with		Fasted Blood Glucose Fold Increase over the Starting Point After 4 weeks)
	λ_{max}	
Cr-picolinate	504 nm	2.37 (95.56%)
Chromex	569 nm	1.88 (75.80%)
CRC 5	531.5 nm	2.15 (86.70%)
CRC 3R	540.5 nm	1.74 (70.16%)
CRC 3V	558 nm	1.09 (43.95%)
Untreated		2.48 (100%)

Biological activity and Vambda max of selected Cr complexes



Based on the data from table 1, it can be concluded that there is a clear relationship between the lambda max of a Cr complex (i.e., between the strength of the Cr-Ligand Coordination Bond) and its biological activity. The optimal range for an absorption maximum is around 560 nm. It is well known that stronger coordination bonds produce more stable complexes that have lower values of lambda max, while weaker coordination bonds produce less stable complexes that show absorption maxima at higher nm values.

In other words, the stability of a Cr complex is its key chemical/biochemical property that directly reflects onto the biological activity of the same.

If complexes are too stable (Cr picolinate, for example) activity is low, and the same is true if the complexes are very unstable.

That is to say, stable complexes look to be rather bioavailable but once they reach the target cells they do not readily transfer Cr atom to the Cr-specific binding protein(s).

On contrary, weak complexes are unstable and they are not sufficiently bioavailable (decomposing in digestive tract into insoluble Cr-hydroxide that is not bioavailable at all).

Cr complexes of an intermediate stability are both bioavailable and active in target cells since they transfer their Cr ion faster and easier than strong complexes would do.

Another important point is water solubility of a Cr complex. Cr picolinate, Cr niacin and Cr yeast are all water insoluble chemical entities and thus their bioavailability by definition is diminished. Our new Cr complexes are all water soluble, sufficiently stable and bioavailable.

As an example, we mention here our new Cr complex ("Chromex") that is made by a reaction of water soluble brewer's yeast extract and Cr chloride hexahydrate. A typical preparation includes a step in which yeast (*e.g.*, baker's yeast) is grown to a desired density, optionally washed and harvested. The cells are then preferably lysed, and the lysate is cleared (*e.g.*, via centrifugation, filtration, etc.) to produce an aqueous yeast extract. This extract may further be processed (*e.g.*, to remove nucleic acids, or protein, add amino acids, or other modification) or used as prepared in another step where the extract is combined with a nutritionally acceptable ion (here: CrIII; *e.g.*, as CrCl₃ salt, or solution thereof). It is contemplated that the ion will form numerous chemically distinct complexes (*e.g.*, with peptides, lipoproteins, polysaccharides, etc.), and that such complexes are sufficiently stable and water soluble, which is superior to so called Cr yeast that is water insoluble material. Leaching organically bound Cr from Cr yeast is an incomplete process and the Cr bioavailability for Cr yeast is seriously diminished.

In other examples, a broader class of water soluble Cr complexes that have appropriate chemical, spectral and biological properties and that can be prepared by reaction a water soluble Cr (III) species with a water soluble organic matrix (yeast extract and/or autolysate, different protein, peptide, amino acid, nucleoside containing natural extracts (like the malted barley extract and the like), different clear fruit juice concentrates, followed by a final freeze drying process. Thus, contemplated complexes exhibit superior solubility in water as well as an improved association constant (range) to provide biologically active complexes from which the ion can be retrieved under physiological conditions

Further examples of appropriate Cr complexes include heterogeneous Cr complexes (binary and ternary Cr complexes) that contain two, three or more different organic and inorganic ligands. The examples of such complexes are given in the appendix: NEW CHROMIUM COMPLEXES. In this appendix the abbreviated names are given together with short procedures of preparing them. Some of these complexes have the optimal spectral characteristics and their biological examination is ongoing. As an example we mention here PK-1C Cr-Kinetin-Citrate and PK-1 (Cr-Kinetin-Chloride). Both complexes absorb at 564 nm and preliminary in vitro data for PK-1 complex gave at 100 nmole concentration 2.3 fold increase (compared to control) of glucose uptake into L6 muscle cells.

In still further aspects, water soluble yeast extracts may also be used to complex ions other than CrIII, and all nutritionally acceptable ions are considered suitable for use herein.

NEW CHROMIUM COMPLEXES

- 1. PK-1C** (Chromium-mono-Kinetin-Citrate)
- 2. PK-2C** (Chromium-di-Kinetin-Citrate)
- 3. PK-3C** (Chromium-tri-Kinetin-Citrate)

- 4. BAK-1C** (Chromium-mono-Benzyl-Adenine-Citrate)
- 5. BAK-2C** (Chromium-di-Benzyl-Adenine-Citrate)
- 6. BAK-3C** (Chromium-tri-Benzyl-Adenine-Citrate)

- 7. CROA-1C** (Chromium-Citrate-Aminooxyacetate)
 - 7a. CROA-1 (Chromium-mono-Aminooxyacetate) – 1 mmol SB
 - 7b. CROA-2 (Chromium-bis-Aminooxyacetate) – 2 mmol SB
 - 7c. CROA-3 (Chromium-tris-Aminooxyacetate) – 3 mmol SB
 - 7d. CROAK-11 (Chromium-mono-Kinetin-mono-Aminooxyacetate) – 1mmol SB
 - 7e. CROAK-21 (Chromium-bis-Kinetin-mono-Aminooxyacetate) – no SB
 - 7f. CROAK-22 (Chromium-bis-Kinetin-bis-Aminooxyacetate) – no SB
- 8. CROX-1C** (Chromium-Citrate-Oxamate)
 - 8a. CROX-1 (Chromium-mono-oxamate) 1 SB
 - 8b. CROX-2 (Chromium-bis-oxamate) 2SB
 - 8c. CROX-3 (Chromium-tris-oxamate) 3SB

- 9. CROC-1** (Chromium-mono-Citrate)
- 10. CROC-2** (Chromium-di-Citrate)

- 11. CC-2** (Chromium-di-Carnosinate)
- 12. CC-3** (Chromium-tri-Carnosinate)
 - 12a **CC-Violet** (acidic pH)
 - 12b. **CC-Red** (basic pH)

- 13. HEX (HromEx)** (Chromium-chloride in YEX)

Molecular Weights of Starting Materials:

$\text{CrCl}_3 \times 6\text{H}_2\text{O}$ (CC-hh) = 266

Citric Acid mono-hydrate (CA-mh) = 210

Kinetin (K) = 215

Benzyl-Adenine (BA) = 225

Oxamic Acid (OA) = 89

Aminooxyacetic Acid $\times \frac{1}{2} \text{HCl}$ (AOA) = 109

Tri-sodium-citrate $\times 2\text{H}_2\text{O}$ (TSC-dh) = 294

Carnosine (C) = 226

Sodium Bicarbonate (SB) = 84

PREPARATION PROCEDURES:

General Remarks:

- A. Take 1 mmol (266 mg) of CrCl_3 hexahydrate (CC-hh) in all cases.
- B. Take one mmol (210 mg) of citric acid mono-hydrate (CA-mh) in cases 1-9.
- C. The bellow given procedure for PK-1C is general for all Cr preparations (1-12). The only variables are the nature and quantity of applied ligands.
 1. **PK-1C:** Mix 266 mg of CC-hh, 210 mg of CA-mh, and 215 mg (1 mmol) of K in six mL of water. Heat in a glass test tube (at boiling water bath for a short time). Add cautiously 168 mg (2 mmol) of SB (CO_2 evolution!). Heat at the boiling water bath for next 2 hours. Transfer the clear blue-violet solution into a plastic vial (14 mL) and dilute to 10 mL (100 mmol solution of PK-1C).
 2. **PK-2C:** 266 mg CC-hh, 210 mg CA-mh, 430 mg (2 mmol) of K and 84 mg (1 mmol) of SB.

3. Preparation of PK-3C (Chromium-tri-Kinetin-Citrate)

266 mg CC-hh, 210 mg CA-mh and 645 mg (3 mmol) of K (*No SB was added!*) is heated in 40 mL of distilled water at 95°C for two hours. K goes slowly into solution and solution becomes gradually violet. Diluted to 50 mL. Final concentration: 20 mmole. Filter(or decant) from small insoluble part.

4. **BAK-1C:** 266 mg CC-hh, 210 mg CA-mh, 225 mg (1 mmol) of BA, 168 mg SB.
5. **BAK-2C:** 266 mg CC-hh, 210 mg CA-mh , 450 mg (2 mmol) of BA and 84 mg SB.
6. **BAK-3C:** 266 mg of CC-hh, 210 mg CA-mh and 675 mg (3 mmol) of BA
7. **CROA-1C:** 266 mg of CC-hh, 210 mg CA-mh, 109 mg of AOA (1mmol) and 252 mg (3mmol) of SB (add in portions cautiously!)
- 7a. **CROA-1:** 266 mg CC-hh, 109 mg AOA and 84 mg mg SB in ~ 10 mL of water. Heat at 95 C for two hours. Dilute to 10 mL exactly.
- 7c. **CROA-3:** 266 mg CC-hh, 32 mg AOA and 252 mg SB in ~ 10 mL of water. Heat at 95 C for two hours. Dilute to 10 mL exactly.
- 7f. **CROAK-22:** 266 mg CC-hh, 218 mg AOA, and 430 mg K in ~ 10 mL of water. Heat at 95 C for two hours. Dilute to 10 mL exactly.
- D. **CROX-1C:** 266 mg of CC-hh, 210 mg CA-mh, 89 mg OA, and 252 mg SB (see 7.). CROX-8a – 8c (work out the synthetic procedures)
- E. **CROC-1:** 266 mg of CC-hh and 294 mg of TSC-dh (1mmol)
- F. **CROC-2:** 266 mg of CC-hh and 588 mg of TSC-dh (2 mmol)
- G. **CC-2:** 266 mg CC-hh, 452 mg (2 mmol) C and 168 mg of SB (2mmol)

H. **CC-3**: 266 mg CC-hh, and 678 mg (3 mmol) C.

Three Different Procedures:

- a) Dissolve CC-hh in 3 ml water; Dissolve C in three ml water; Mix quickly. Precipitate forms; Heat for a short time and add slowly 252 mg SB. Heat 2 hours at boiling wather bath. Appreciable amount of precipitate stays insoluble on prolonged heating. It looks like Cr-hydroxide. Final pH Basic.
 - b) Dissolve CC-hh in 6 ml water. Heat at the boiling water bath for a short time. Add slowly to hot solution solid C. No precipitate forms. Heat for another 10 minutes and then add slowly and cautiously 160 mg SB. No precipitate froms. On prolonged heating (two to three hours) the solution satys clear. The final color is in between red and violet, closer to red. It is named: **CC-3R (12b)**. Final pH close to neutral.
 - c) **Dissolve CC-hh in water (6 ml). Heat and add slowly C. No precipitate! Do not add SB!! Heat for next 2 hours at the boiling water bath. Stays clear! Stays slightly acidic. Color: Violet. It is named: CC-3V (12a).**
- I. **HEX**: 266 mg CC-hh, 400 mg (AMBEREX)) in 10 mL water. Keep 2 hours at RT. Filter from small insoluble discarded part.

REMARK:

12 a: CC-3 Violet, water soluble (the most acidic pH). Taken for animal experiment at Ray's Lab. **13., HEX**, taken for animal experiment. **1., PK-1C**, taken for animal experiment.

12 b : CC-3 Red(-violet), water soluble (pH close to neutral). Should be compared to CC-3V in the second experiment with Ray M.

Compounds in "violet font" are tested in the first experiment (at Ray's Lab, on streptozocin-induced diabetes rats).

All compounds in "blue font" will be tested in vitro by ZB. In addition, CC-3R will be tested in second experiment with Ray.

Total glucose uptake in L6 muscle cells in vitro induced by various chromium compounds. Summary of screening results

San Diego, September 08,

2003

Experiments were performed in FutureCeuticals, Inc., 5080 Shoreham PL, Ste 205, San Diego, CA 92122.

Compounds	Conc. nM	Fold over Control	Range	Average
CrCl3	10	1.44, 1.23, 0.95, 3.38, 0.94, 1.50, 1.44, 2.1, 1.3, 1.34, 1.46	0.94-3.38	1.55
	100	1.46, 1.33, 4.05, 2.0, 2.44, 1.56, 2.30, 1.52, 1.93	1.33-4.05	2.05
	1000	1.64, 1.24, 3.05, 2.83, 2.76, 1.68, 1.60, 1.63, 2.42	1.24-3.05	2.08
CRX/Hex	10	4.44, 1.66, 3, 1.70, 2.40, 1.50, 1.40, 1.51	1.40-4.44	2.15
	100	4.88, 3.94, 2.75, 2.01, 4.50, 2.40, 1.84, 2.29	1.84-4.88	3.07
	1000	3.83, 4.26, 4.10, 2.44, 3.90, 3.3 2.39, 1.94	1.94-4.26	3.26
CRC3R	10	3.5, 3.0, 2.5, 1.6	1.6-3.5	2.65
	100	4.5, 4.0, 4.5, 3.0,	3.0-4.5	4.00
	1000	6.0, 2.60, 3.20, 2.0,	2.0-6.0	3.45
CRC3V	10	4.70, 1.88, 2.0, 3.0, 1.71	1.71-4.70	2.65
	100	8.50, 2.20, 4.9, 2.2,	2.20-8.50	4.45
	1000	4.10, 2.90, 3.00, 3.40, 2.10	2.10-4.10	3.10
CRC5	10	2.50, 1.77, 1.60, 1.30	1.30-2.50	1.73
	100	3.17, 6.10, 3.2, 2.10,	2.10-6.10	3.57
	1000	3.20, 4.50, 2.60, 2.60	2.60-4.50	3.22
PK-1	10	1.15, 1.60, 1.56, 1.39, 1.30, 3.88, 2.88	1.15-3.88	1.96
	100	1.28, 1.60, 1.71, 1.98, 2.0, 5.10	1.28-5.10	2.28
	1000	1.61, 1.51, 1.92, 1.63, 1.83, 2.27,	1.51-2.27	1.79
PK3	10	1.26, 1.39, 1.49, 1.40, 2.36, 1.50, 1.38	1.26-2.36	1.54

	100	1.50, 1.0, 2.42, 3.45, 2.83	1.0-3.45	2.24
	1000	1.38, 1.0, 1.50, 2.37, 3.33	1.0-3.33	1.91
CROA -1	10	1.41, 1.66, 2.10,	1.41-2.10	1.72
	100	2.12, 2.16, 2.80,	2.12-2.80	2.36
	1000	2.58, 2.10, 2.50	2.10-2.58	2.39
CROA-1C	10	1.08, 1.07, 1.44,	1.07-1.44	1.19
	100	1.32, 1.35, 1.90	1.32-1.90	1.52
	1000	1.48, 1.96, 2.06	1.48-2.06	1.83
CROA-3	10	1.46, 1.16, 1.79	1.16-1.79	1.47
	100	1.78, 1.38, 1.78	1.38-1.78	1.64
	1000	2.12, 1.75, 2.02,	1.75-2.12	1.96
OA-K1	10	1.98, 3.0, 1.96, 1.72, 2.20	1.72-3.00	2.16
	100	2.52, 3.07, 2.68, 2.28, 1.99	1.99-3.07	2.50
	1000	2.07, 2.09, 2.60, 2.52, 1.60	1.60-2.60	2.17
MTF	10uM	2.16, 2.30, 2.80, 2.26	2.16-2.80	2.38
	100	3.59, 5.0, 3.0, 3.0, 2.0, 2.24, 2.57	2.0-5.0	3.10
	1000	2.0, 2.45, 2.04	2.04-2.45	2.16

Method: Total glucose uptake was measure using fluorescent analog of glucose, 2-NBDG from Molecular Probes Inc. L6 myoblastic cells were treated for 2 hrs with tested compounds in culture medium SkBM from Clonetics. After washing, cells were transferred to HBSA (Hepes-buffered Saline), pH 7.0 with 50uM of 2-NBDG without glucose. One minute later, cells were washed with ice-cold PBS, and fixed in -20C 70% ethanol. Fluorescence was measured at 480/530 (excitation/emission).

Activity of selected chromium compounds in vivo.

Model: Streptozocin-induced insulin deficient rats (commonly known as streptozocin – induced diabetes type I rats).

Streptozocin causes damage of pancreas resulting in drastically reduced secretion of insulin. As consequence, these rats develop severe hyperglycemia.

So far, chromium was known to potentate action of insulin, regardless that exact mechanism was not known. Recently, chromium was found to stimulate AKT thus inducing glucose uptake to muscle cells in insulin-independent way. Still, activation of AKT may justified why chromium was recognized for long time as insulin enhancer.

In our studies in vivo, chromium compounds were provided in drinking water for four weeks at dose 42ug/kg. Vein blood was collected following four hrs fasting and used for fasted blood glucose level test.

Preliminary results from two independent studies in vivo

Study I

Compound	Fasted Blood Glucose Level [Fold increase over 4 weeks]
Untreated	1.95
CrCl ₃	1.32
CRC3-V	0.80
CRC 5	1.02
CrPic	2.02
Chromex	1.55
CrNiacin	1.91
Metformin	0.96

Study II*

Untreated	2.48
CrPic	2.37
Chromex	1.88
CRC 3-R	1.74
CRC3-V	1.09
CRC5	2.15

* study is still ongoing and results are based on three weeks of treatment only

These results show quite dramatic improvement of glucose transport in insulin-deficient rats. These rats are hypo-insulinemic and hyper-glycemic due to severe pancreatitis conditions. Improvement under such conditions indicate that the treatment overpass insulin-deficiency and stimulate glucose utilization.

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